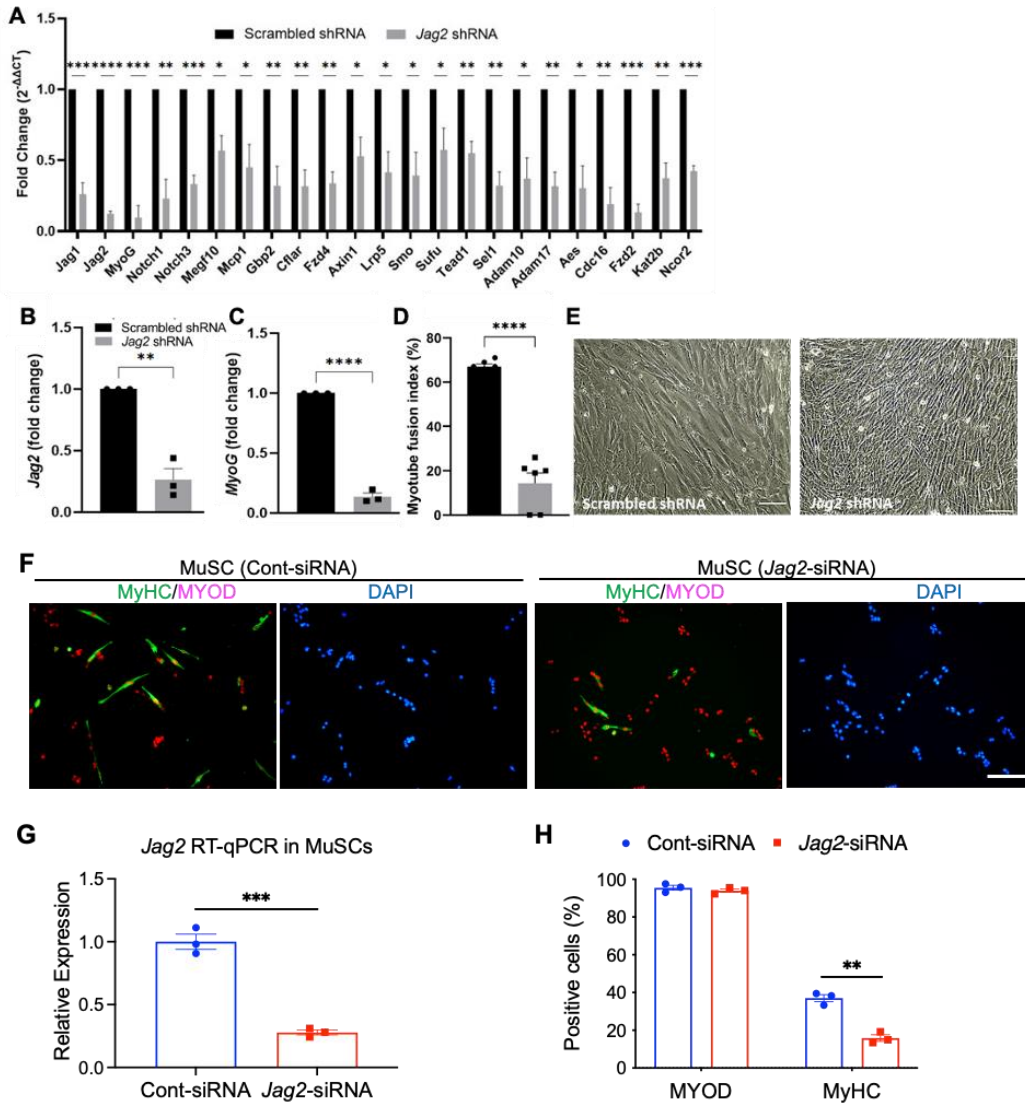
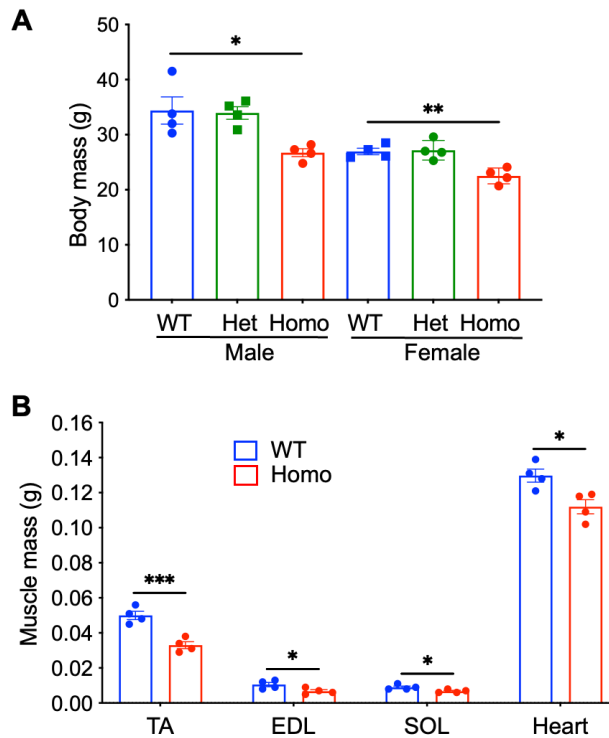


# Figure S1



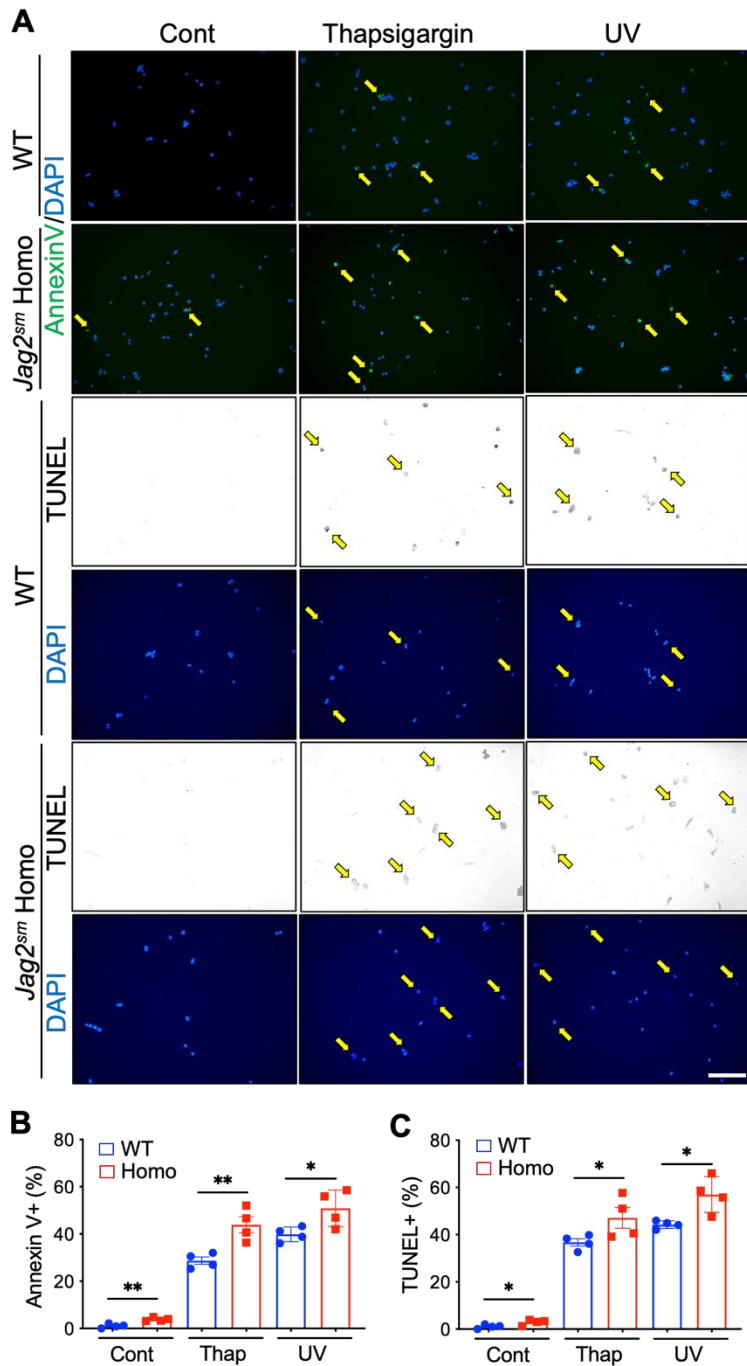
**Figure S1. Impaired myogenic differentiation and Notch signaling in *JAG2* deficient-myoblasts.** (A) *Jag2* shRNA and scrambled shRNA C2C12 myoblasts were differentiated for 7 days. RT-qPCR was performed on isolated RNA with an array plate containing probes for 93 Notch signaling and myogenesis genes, along with 3 control genes. The 23 significantly downregulated genes are shown in the graph, including *MyoG*, *Notch1*, and *Notch3*. No genes were significantly upregulated. Transcript levels were normalized to *Gapdh*. (B-D) The down-regulated genes were confirmed by RT-qPCR. (E) Phase contrast images of day 7-differentiated *Jag2* knockdown cells show a lack of myotubes compared to scrambled shRNA cells. (F) WT MuSCs were transfected with control or *Jag2* siRNA, cultured in differentiation medium for 1 day and stained with MYOD (red) and MyHC(+) for myogenic differentiation (green). DAPI stained all nuclei (blue). (G) *Jag2* expression was assessed by RT-qPCR following transfection with control or *Jag2*-siRNA. Transcript levels were normalized to *18S*. (H) Number of nuclei harboring MyHC- or MYOD-positive cells on day 1 of differentiation in control cells and *Jag2*-siRNA-treated cells. Scale bars, 100  $\mu$ m. Student's t-tests showed \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  and \*\*\*\* $p < 0.0001$ . Error bars show SEM [(A, B, C, G, H)  $n = 3$ , and (D)  $n = 5$  biological replicates].

# Figure S2



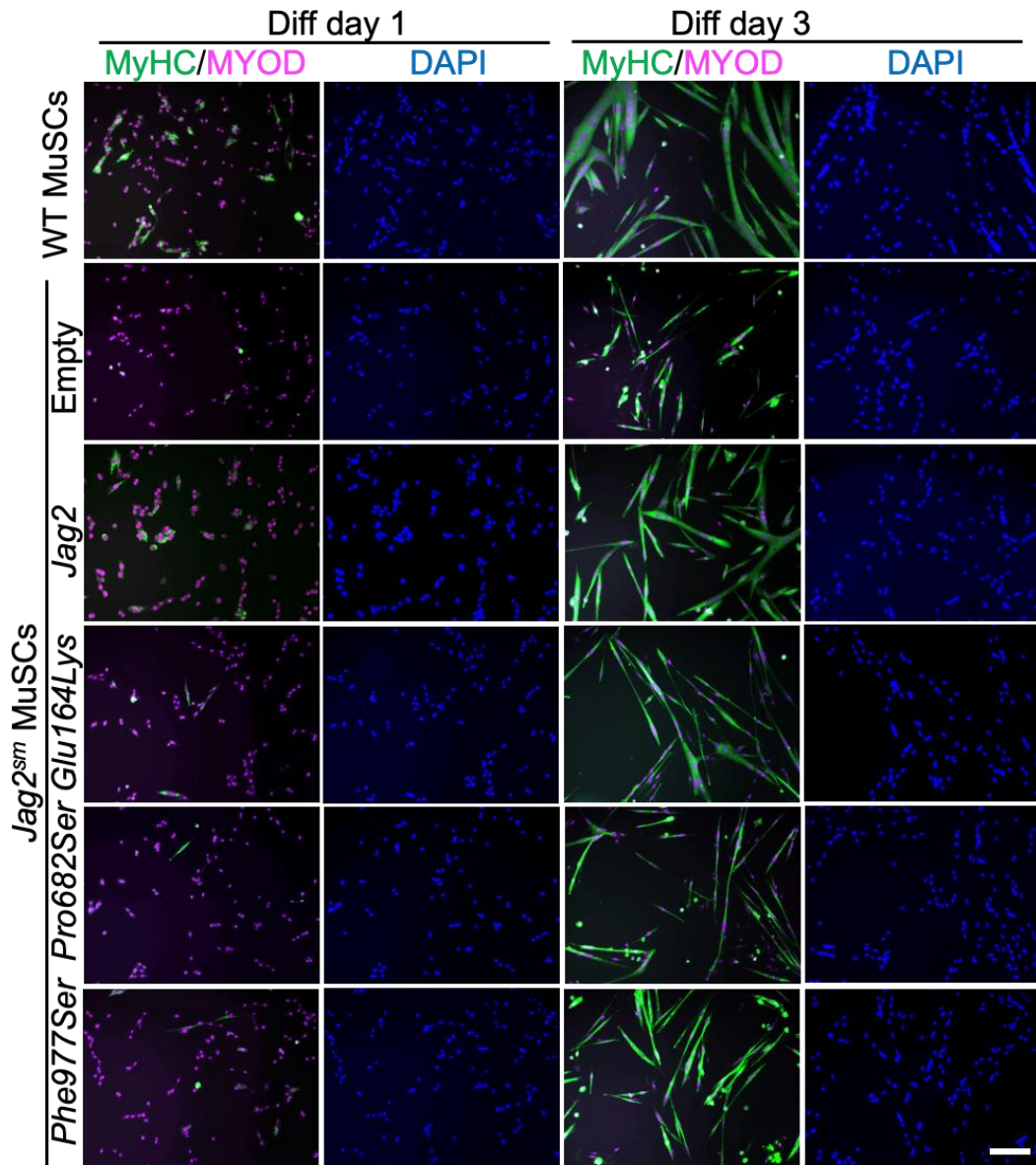
**Figure S2. Reduced body and muscle mass in homozygous *Jag2<sup>sm</sup>* mice.** Three-month-old *Jag2<sup>sm</sup>* homozygous male and female mice showed reduced body mass vs. *Jag2<sup>sm</sup>* WT and/or heterozygous mice. Three-month-old *Jag2<sup>sm</sup>* homozygous male tibialis anterior (TA), extensor digitorum longus (EDL), soleus (SOL) muscle, and heart showed reduced muscle mass vs. *Jag2<sup>sm</sup>* WT mice. Student's t-tests showed \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ . Error bars show SEM (n=4 biological replicates).

# Figure S3



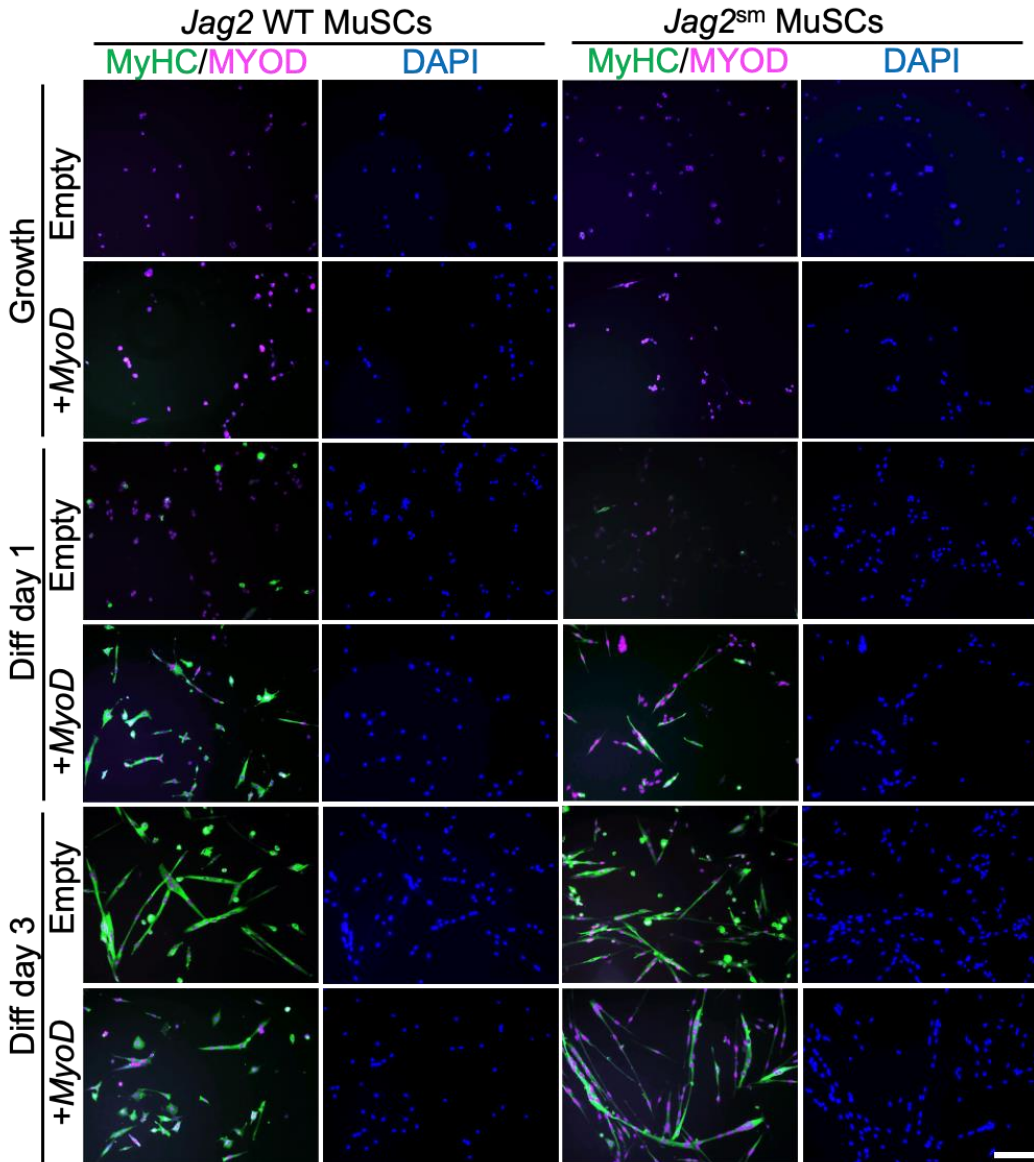
**Figure S3. *Jag2<sup>sm</sup>* MuSCs show increased apoptotic cell death.** MuSCs isolated from WT and homozygous *Jag2<sup>sm</sup>* mice were used for detection of apoptosis [arrows, Annexin V(+) green-stained cells or TUNEL(+) gray-stained cells]. Twenty-four hours following treatment with thapsigargin or UV exposure. DAPI stained all nuclei (blue). Scale bars, 100  $\mu$ m. Student's t-tests showed \* $p < 0.05$  and \*\* $p < 0.01$ . Error bars show SEM ( $n = 4$  biological replicates).

# Figure S4



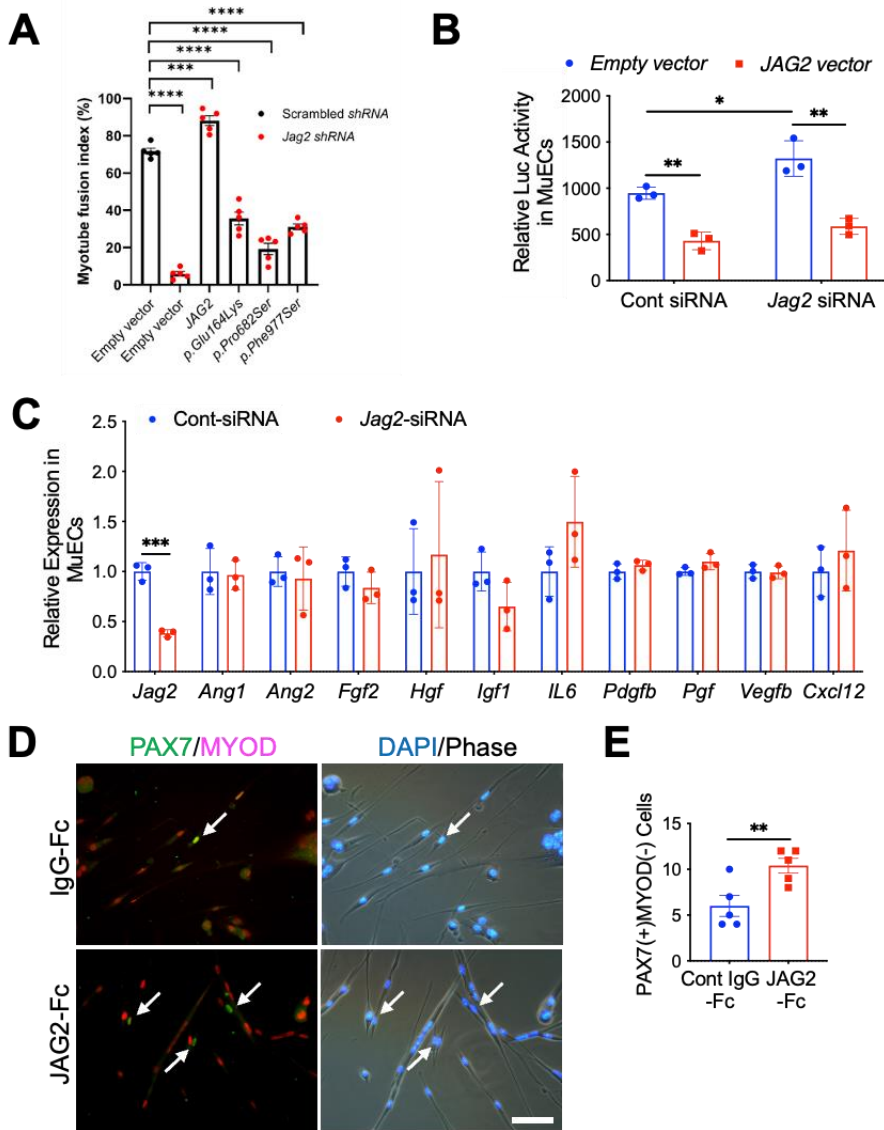
**Figure S4. Overexpression of JAG2 rescues differentiation defects in *Jag2<sup>sm</sup>* MuSCs.** MuSCs isolated from WT and homozygous *Jag2<sup>sm</sup>* mice were used for overexpression of human reference JAG2 (*Jag2<sup>WT</sup>*) and human JAG2 harboring three patient variants (*p.Glu164Lys*, *p.Pro682Ser*, and *p.Phe977Ser*). Overexpression of *Jag2<sup>WT</sup>* but not JAG2 variants increased expression and MyHC(+) myogenic differentiation (green) and rescued myogenic differentiation defects in *Jag2<sup>sm</sup>* MuSCs. MYOD expression was shown in nuclei (purple). DAPI stained all nuclei (blue). Scale bars, 100  $\mu$ m.

# Figure S5



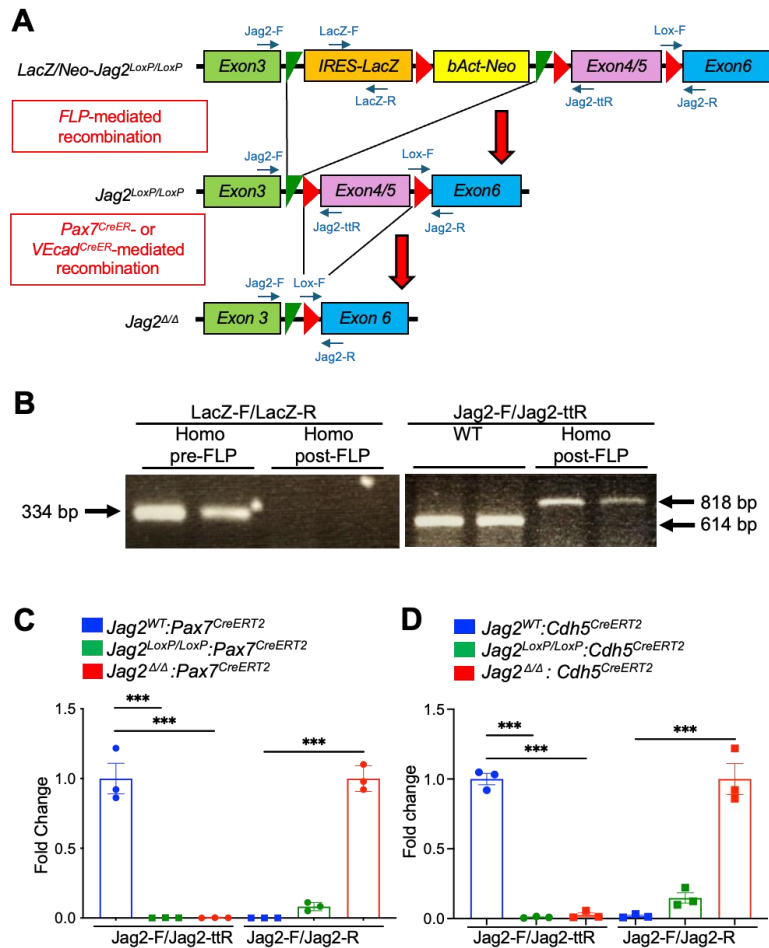
**Figure S5. *MyoD* rescue myogenic differentiation in *Jag2<sup>sm</sup>* MuSCs and direct binding of *Jag2* to *Notch1-3*.** MuSCs isolated from WT and homozygous *Jag2<sup>sm</sup>* mice were used for overexpression of *MyoD*. Overexpression of *MyoD* increased MYOD (purple) expression and MyHC(+) myogenic differentiation (green) and rescued myogenic differentiation defects in *Jag2<sup>sm</sup>* MuSCs. DAPI stained all nuclei (blue). Scale bars, 100  $\mu$ m.

# Figure S6



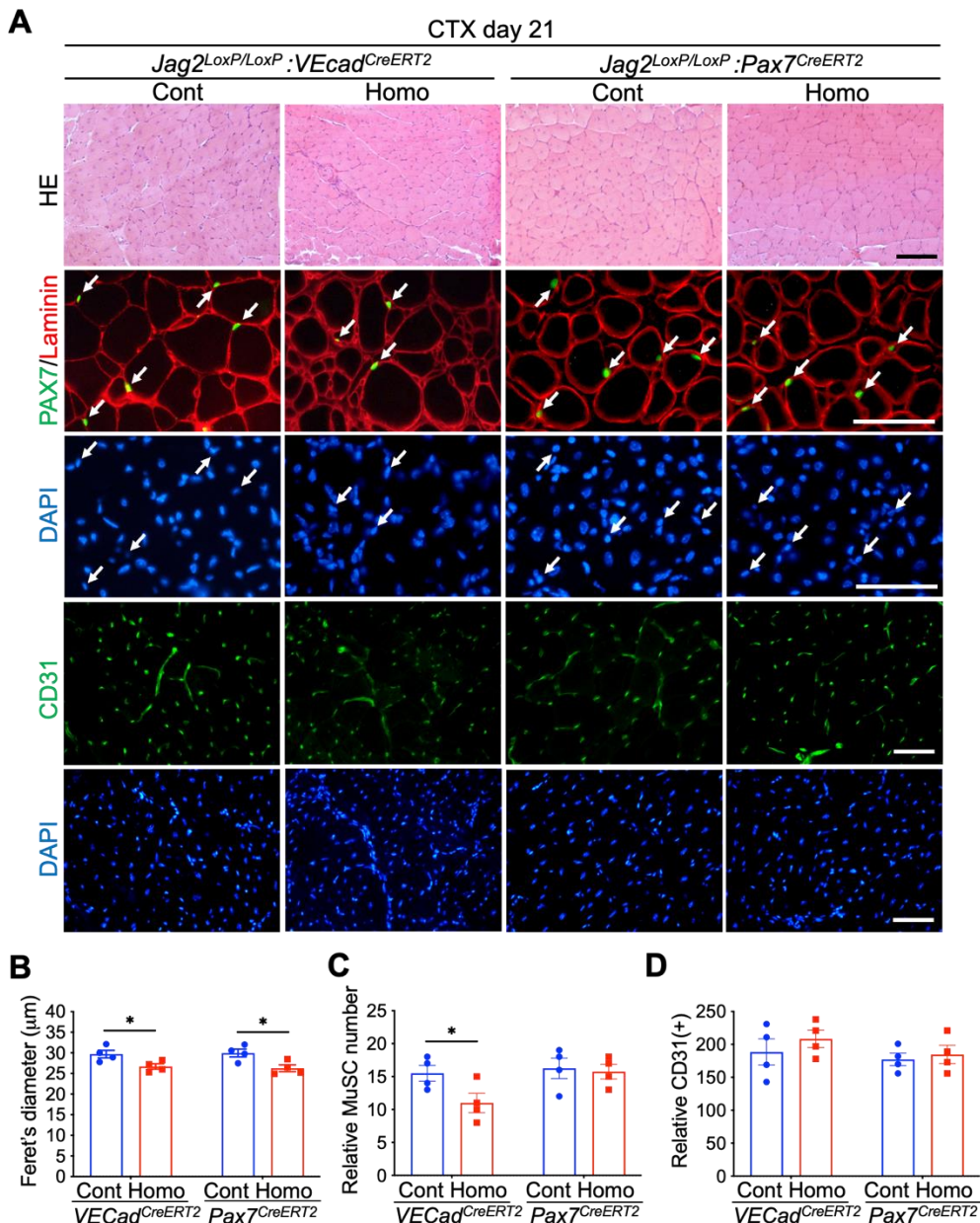
**Figure S6. Effects of *Jag2* KD and overexpression in MuECs and MuSCs.** (A) *Jag2* shRNA cells with stable overexpression of empty vector, *JAG2* and three patient variants were differentiated for 6 days. Scrambled shRNA cells was used for the control. Following MyHC staining, myotube fusion index was quantified. *Jag2* knockdown reduced the myotube fusion but overexpression of *JAG2* but not the variants rescued this defect. (B) MuECs were stably transfected with empty vector or *JAG2* expression vector. In MuECs, overexpression of *JAG2* decreased *Hes1-467-Luc* activity compared with the empty vector following transfection. Transfection with siRNA for *Jag2* increased *Hes1-467-Luc* activity compared with control scramble siRNA. (C) Gene expression of *Jag2* and secreted molecules involved in MuSC proliferation and differentiation were compared between MuECs with control scramble siRNA and siRNA for *Jag2*. (D, E) PAX7(+)MYOD(-) reserve cells (arrows) were assessed in MuSCs exposed to control-IgG-Fc or the extracellular *JAG2*-Fc for 5 days in differentiation medium. DAPI stained all nuclei (blue). Scale bar, 100  $\mu$ m. One-way Anova followed by the Bonferroni post hoc tests showed \*\*\* $p < 0.001$  and \*\*\*\* $p < 0.0001$ . Student's t-tests showed \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ . Error bars show SEM [(A, E)  $n = 5$ , and (B, C)  $n = 3$  biological replicates].

# Figure S7



**Figure S7. Confirmation of conditional deletion of *Jag2*.** (A) Schematic genomic structure of *Jag2*<sup>flxed</sup> locus for conditional *Jag2* mutant (*LacZ/Neo-Jag2*<sup>LoxP/LoxP</sup>) mice. Following breeding with *FLP* mice, Flippase-Frt sites (green triangles)-mediated *IRES-LacZ/bAct-Neo* cassette deletion occurred. Following breeding with *Pax7*<sup>CreER</sup> or *VEcad*<sup>CreER</sup> mice and TMX-treatment, *CreER-LoxP* sites (red triangles)-mediated *Exons 4/5* deletion occurred. (B) Genomic PCR was performed to detect the *LacZ* gene in pre-*FLP* but not in post-*FLP* mice in homozygous *LacZ/Neo-Jag2*<sup>LoxP/LoxP</sup> mice using *Lac-F/LacZ-R* primers. Following breeding with *FLP* mice, an 818 bp band appeared in homozygous *LacZ/Neo-Jag2*<sup>LoxP/LoxP</sup> mice while WT mice showed a 614 bp band using *Jag2-F/Jag2-ttR* primers. (C) MuSCs isolated from *Jag2*<sup>LoxP/LoxP</sup>;*Pax7*<sup>CreER</sup> (without TMX) or *Jag2*<sup>Δ/Δ</sup>;*Pax7*<sup>CreER</sup> (with TMX) mice showed no DNA amplification by qPCR using *Jag2-F/Jag2-ttR* primers, while MuSCs from *Jag2*<sup>WT</sup>;*Pax7*<sup>CreER</sup> mice showed the DNA amplification. Following treatment with TMX, qPCR using *Jag2-F/Jag2-R* primers amplified the DNA in MuSCs from *Jag2*<sup>Δ/Δ</sup>;*Pax7*<sup>CreER</sup> mice, while *Jag2*<sup>WT</sup>;*Pax7*<sup>CreER</sup> or *Jag2*<sup>LoxP/LoxP</sup>;*Pax7*<sup>CreER</sup> mice showed no DNA amplification. (D) MuECs from *Jag2*<sup>LoxP/LoxP</sup>;*VEcad*<sup>CreER</sup> (without TMX) or *Jag2*<sup>Δ/Δ</sup>;*VEcad*<sup>CreER</sup> (with TMX) mice showed no DNA amplification by qPCR using *Jag2-F/Jag2-ttR* primers, while *Jag2*<sup>WT</sup>;*VEcad*<sup>CreER</sup> showed DNA amplification. Following treatment with TMX, qPCR using *Jag2-F/Jag2-R* primers amplified DNA in MuECs from *Jag2*<sup>Δ/Δ</sup>;*VEcad*<sup>CreER</sup> mice but not in *Jag2*<sup>WT</sup>;*VEcad*<sup>CreER</sup> or *Jag2*<sup>LoxP/LoxP</sup>;*VEcad*<sup>CreER</sup> mice. One-way Anova followed by the Bonferroni post hoc tests showed \*\*\**p*<0.001. Error bars show SEM (n=3 biological replicates).

# Figure S8



**Figure S8. Reduced self-renewal in *Jag2<sup>LoxP/LoxP</sup> : VECad<sup>CreERT2</sup>* and reduced regeneration in *Jag2<sup>LoxP/LoxP</sup> : Pax7<sup>CreERT2</sup>* mice.** (A) Following TMX injection, CTX injections into the TA were performed in *Jag2<sup>LoxP/LoxP</sup> : VECad<sup>CreERT2</sup>* and *Jag2<sup>LoxP/LoxP</sup> : Pax7<sup>CreERT2</sup>* mice. H&E, anti-PAX7 (green, arrows)/Laminin (red) and anti-CD31 (green) antibody staining were performed by 21 days following CTX injection. DAPI stained all nuclei (blue). (B) Feret's diameters of TA muscle fibers, (C) anti-PAX7(+) MuSCs, and (D) CD31(+) capillaries were counted. Scale bars, 100 µm. Student's t-tests showed \**p*<0.05. Error bars show SEM (*n*=4 biological replicates).